1. Inoculate 5 ml LB/ampicillin (50 µg/ml) medium placed in a 10-20 ml culture tube with *E. coli* carrying desired plasmid and grow at 37 °C with agitation for 12-16 h.
2. Pellet 1.5-5 ml bacteria in appropriate vessels by centrifugation at 10,000 *x g* for 1 min at room temperature.
3. Decant or aspirate medium and discard. To the bacterial pellet add 250 µl solution I/RNase A.
4. Add 250 µl solution II and mix gently but thoroughly by inverting and rotating tube 4-6 times to obtain a cleared lysate.
5. Add 125 µl ice-cold buffer N3 and mix gently but thoroughly by inverting tube several times until a flocculent white precipitate forms.
6. CAREFULLY aspirate and transfer the cleared supernatant to a clean 1.5 ml centrifuge tube. Add 0.1 volume of ETR solution to the cleared lysate.
7. Incubate the lysate at 42 °C for 5 min. The lysate should appear turbid again. Centrifuge at 12,000 *x g* for 3 min at 25 °C. The ETR solution will form a blue layer at bottom of tube.
8. Transfer the top aqueous phase (cleared lysate) into a new 1.5 ml tube and add 0.5 volume of absolute ethanol (room temperature, 96-100%) and gently mix by inverting tube 6-7 times.
9. Transfer 700 µl of the mixture (from step 8) into a clean HiBindTM DNA Mini Column assembled in a 2 ml collection tube (provided).
10. Discard the flow-through and load the remaining of the mixture into the column and centrifuge as above. Discard the flow-through and re-use the collection tube.
11. Wash column with 500 µl buffer HB and centrifuge as above.
12. Discard the flow-through liquid and wash the column by adding 700 µl DNA wash buffer diluted with ethanol. Centrifuge as above and discard flow-through.
13. Repeat wash step 12 with another 700 µl DNA wash buffer diluted with ethanol.
14. Discarded the flow-through liquid.
15. Place column into a new clean 1.5 ml micro-centrifuge tube. Add 30-50 µl (depending on desired concentration of final product) endotoxin-free elution buffer (or water) directly onto the column matrix and centrifuge at 13,000 *x g* for 1 min to elute DNA.